

Serial No 09/730,525, filed December 5, 2000
Docket No. 20757USDIV6

Amendments to the Claims

This listing of claims will replace all prior versions and listing of claims in the application.

Listing of Claims

Claims 1-14 (Canceled).

15. (original) A method for forming a mutagenized DNA molecule encoding an enzyme having protox activity from at least two non-identical template DNA molecules encoding enzymes having protox activity, said method comprising the steps of:

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- a) adding to the template DNA molecules at least one oligonucleotide comprising an area of identity to each of the template DNA molecule;
 - b) denaturing the resultant mixture into single-stranded molecules;
 - c) incubating the resultant population of single-stranded molecules with a polymerase under conditions that result in the annealing of the oligonucleotides to the template DNA molecules, wherein the conditions for polymerization by the polymerase are such that polymerization products corresponding to a portion of the template DNA molecules are obtained;
 - d) repeating the second and third steps for at least two further cycles, wherein the extension products obtained in step c) are able to switch template DNA molecule for polymerization in the next cycle, thereby forming a mutagenized double-stranded polynucleotide comprising sequences derived from different template DNA molecules;

wherein the mutagenized double-stranded polynucleotide encodes a protox enzyme having enhanced tolerance to a herbicide that inhibits the protox activity encoded by the template DNA molecules.

16. (original) The method of claim 15, wherein at least one template DNA molecule is derived from a eukaryote.

17. (original) The method of claim 16, wherein said eukaryote is a higher eukaryote.

18. (original) The method of claim 17, wherein said higher eukaryote is a plant.

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19. (original) The method of claim 17, wherein said plant is selected from the group consisting of *Arabidopsis thaliana*, oilseed rape, soybean, sugarbeet, cotton, maize, wheat, rice, sugarcane, and sorghum.

20. (original) The method of claim 17, wherein at least one said template DNA molecule derived from said plant comprises at least one mutation and encodes a modified protoporphyrinogen oxidase (protox) having at least one amino acid modification, wherein said modified protox is tolerant to a herbicide in amounts that inhibit said protox.

21. (amended once) The method of claim 20, wherein at least one said template DNA molecule is further characterized in that at least one of the following conditions is met:

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- (a) said template DNA molecule has a sequence that encodes amino acid sub-sequence $AP\Delta_1F$, wherein Δ_1 is an amino acid other than arginine;
 - (b) said template DNA molecule has a sequence that encodes amino acid sub-sequence $F\Delta_2S$, wherein Δ_2 is an amino acid other than cysteine;
 - (c) said template DNA molecule has a sequence that encodes amino acid sub-sequence $Y\Delta_3G$, wherein Δ_3 is an amino acid other than alanine;
 - (d) said template DNA molecule has a sequence that encodes amino acid sub-sequence $A\Delta_4D$, wherein Δ_4 is an amino acid other than glycine;
 - (e) said template DNA molecule has a sequence that encodes amino acid sub-sequence $Y\Delta_5P$, wherein Δ_5 is an amino acid other than proline;
 - (f) said template DNA molecule has a sequence that encodes amino acid sub-sequence $P\Delta_6A$, wherein Δ_6 is an amino acid other than valine;
 - (g) said template DNA molecule has a sequence that encodes amino acid sub-sequence Δ_7IG , wherein Δ_7 is an amino acid other than tyrosine;
 - (h) said template DNA molecule has a sequence that encodes amino acid sub-sequence $YIGG\Delta_8$, wherein Δ_8 is an amino acid other than alanine or serine;
 - (i) said template DNA molecule has a sequence that encodes amino acid sub-sequence $A\Delta_9P$, wherein Δ_9 is an amino acid other than isoleucine;
 - (j) said template DNA molecule has a sequence that encodes amino acid sub-sequence $G\Delta_{10}A$, wherein Δ_{10} is an amino acid other than valine;

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(k) said template DNA molecule has a sequence that encodes amino acid sub-sequence $Y\Delta_3G$, wherein Δ_3 is an amino acid other than alanine, and said template DNA molecule also has a sequence that encodes one of the group consisting of:

- (1) sub-sequence $Q\Delta_{11}S$, wherein Δ_{11} is an amino acid other than proline,
- (2) sub-sequence $IGG\Delta_{12}$, wherein Δ_{12} is an amino acid other than threonine,
- (3) sub-sequence $SWXL\Delta_{13}$, wherein Δ_{13} is an amino acid other than serine,
- (4) sub-sequence $L\Delta_{14}Y$, wherein Δ_{14} is an amino acid other than asparagine, and
- (5) sub-sequence $G\Delta_{15}XGL$, wherein Δ_{15} is an amino acid other than tyrosine;

(l) said template DNA molecule has a sequence that encodes amino acid sub-sequence Δ_7IG , wherein Δ_7 is an amino acid other than tyrosine, and said template DNA molecule also has a sequence that encodes one of the group consisting of:

- (1) sub-sequence $Q\Delta_{11}S$, wherein Δ_{11} is an amino acid other than proline,
- (2) sub-sequence $IGG\Delta_{12}$, wherein Δ_{12} is an amino acid other than threonine,
- (3) sub-sequence $SWXL\Delta_{13}$, wherein Δ_{13} is an amino acid other than serine,
- (4) sub-sequence $L\Delta_{14}Y$, wherein Δ_{14} is an amino acid other than asparagine, and
- (5) sub-sequence $G\Delta_{15}XGL$, wherein Δ_{15} is an amino acid other than tyrosine; and

(m) said template DNA molecule has a sequence that encodes amino acid sub-sequence $T\Delta_{16}G$, wherein Δ_{16} is an amino acid other than leucine, and said template DNA molecule also has a sequence that encodes amino acid sub-sequence $YV\Delta_{17}G$, wherein Δ_{16} is an amino acid other than alanine.

22. (original) The method of claim 15, wherein at least one said template DNA molecule is derived from a prokaryote.

23. (original) The method of claim 15, wherein said herbicide is selected from the group consisting of an aryluracil, a diphenylether, an oxidiazole, an imide, a phenyl pyrazole, a pyridyl pyrazole, a pyridine derivative, a 3-substituted-2-aryl-4,5,6,7-tetrahydroindazole, a phenopylate and O-phenylpyrrolidino- and piperidinocarbamate analogs of said phenopylate.

Claims 24-25 (Canceled).

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26. (Previously presented) A construct comprising the following components in the 5' to 3' direction of transcription:

- a) a promoter functional in a plant plastid;
- b) a DNA sequence encoding a peptide derived from an eukaryotic organism; and
- c) a transcription termination region; and

the construct further comprising a ribosome binding site joined to said promoter component, said ribosome binding site heterologous to said promoter;

wherein said DNA sequence encoding a eukaryotic peptide is heterologous to a plant plastid.

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27. (Previously presented) The construct according to claim 26 wherein said DNA sequence encodes a plant nuclear peptide.

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28. (Previously presented) A plant cell plastid containing the construct according to claim 26.

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29. (Previously presented) A plant, plant seed, plant cell or progeny thereof each containing a plant plastid according to claim 28.

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30. (Previously presented) A method for producing a protein in a plant cell, wherein said method comprises transforming plastids of said plant cell with a construct comprising the following as operably joined components in the 5' to 3' direction of transcription:

- (a) a promoter functional in a plant plastid;
- (b) a DNA sequence encoding a peptide of an eukaryotic cell other than a peptide of a plant plastid; and
- (c) a transcription termination region,

the construct further comprising a ribosome binding site joined to said promoter, said ribosome binding site heterologous to said promoter;

wherein said DNA sequence encoding a peptide of an eukaryotic cell is heterologous to a plant plastid;

and growing plant cells comprising said transformed plastids under conditions wherein said DNA sequence is expressed to produce said eukaryotic peptide in said plastid.

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31. (Previously presented) A plant cell having a transformed plastid produced according to the method of claim 30.

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32. (Previously presented) A plant, plant seed or plant part each comprising a plant cell according to claim 31.

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33. (Previously presented) The method according to claim 30 wherein said eukaryotic peptide encoded by said DNA sequence from said construct in said transformed plant plastid is bioactive when isolated from said transformed plant plastid.

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34. (New) A nucleic acid molecule comprising a chimeric gene, said chimeric gene comprising: a promoter, a sequence from a 5' UTR, and a 3' UTR each operatively linked to a eukaryotic gene, wherein said promoter is functional in a plant plastid and is heterologous to said 5' UTR, and wherein said eukaryotic gene is heterologous to a plant plastid and is expressed in a plant plastid.

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35. (New) The molecule according to claim 34, wherein said 5' UTR is derived from plastid DNA.

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36. (New) The molecule according to claim 35, wherein said 5' UTR is from the psbA gene.

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37. (New) The molecule according to claim 34 wherein said eukaryotic gene encodes a plant nuclear peptide.

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38. (New) A plant cell plastid containing the molecule according to claim 34.

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39. (New) A plant, plant seed, plant cell or progeny thereof each containing a plant plastid according to claim 38.

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40. (New) A method for producing a protein in a plant cell comprising: transforming a plastid of said plant cell with a molecule comprising a chimeric gene, said chimeric gene comprising: a promoter, a sequence from a 5' UTR, and a 3' UTR each operatively linked to a eukaryotic gene, wherein said promoter is functional in a plant plastid and is heterologous to said 5' UTR, and wherein said eukaryotic gene is heterologous to a plant plastid and is expressed in a plant plastid; and growing plant cells comprising said transformed plastids under conditions wherein said DNA sequence is expressed to produce said eukaryotic peptide in said plastid.

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41. (New) The method according to claim 40, wherein said 5' UTR is from a plastid gene.

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42. (New) A plant cell having a transformed plastid produced according to the method of claim 40.

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43. (New) A plant, plant seed or plant part each comprising a plant cell according to claim 42.

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44. (New) The method according to claim 40 wherein said eukaryotic peptide encoded by said DNA sequence from said construct in said transformed plant plastid is bioactive.
